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THE STRUCTURE AND DEVELOPMENT OF THE SPOROPHYLLS AND SPORANGIA OF ISOETES.

CONTRIBUTION FROM THE HULL BOTANICAL LABORATORY. XVIII.

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(WITH PLATES XIII–XX)

FEW plants have excited more interest than Isoetes, a small genus of about fifty species, which has been variously classified, and the histology and development of which have been described in the most contradictory manner. It was with the purpose of obtaining, if possible, some data by which to clear up its homologies and relationships, and especially of examining the foundation of a claim made in recent years of its being the point of contact between monocotyledons and vascular cryptogams that the following investigation was undertaken.

The intention at first was to have the work include not only the reproductive parts of the sporophyte, but also the development of the female gametophyte and of the embryo. But so small a proportion of the spores was found capable of germination that the study of the prothallium had to be abandoned; and my

observations of the embryo agree so closely with those of Professor Campbell (4) that it did not seem worth while to publish any drawings. One difference may be noticed here and this part of the subject may be dismissed at once. Campbell says that but three archegonia are formed at first, and only in case none of these are fertilized do others appear. I have found new archegonia appearing even after three embryos had begun to develop, two of which had made considerable growth. It thus appears that sometimes new archegonia may arise even after fertilization.

The species selected for study were *I. echinospora* and *I. Engelmanni*, the former of which was examined more carefully. The material was collected by Mr. Raynal Dodge, of Newburyport, Mass. Part of it was fixed at once, and part after it had been cultivated for some time in the laboratory. The fixing reagents employed were 1 per cent. chrom-acetic acid, and Flemming's weaker solution. After remaining twenty-four hours in one of these fluids it was washed twenty-four hours in water and transferred through graded alcohols and chloroform or xylol to paraffin. The sections were cut 5, 10, or 15μ in thickness and stained in the ordinary way on the slides. Delafield's hæmatoxylin and erythrosin, safranin and gentian violet, Heidenhain's iron-alum-hæmatoxylin, and cyanin and erythrosin were all used with good results except in the case of the megaspore mother cell.

THE STEM.

The technique which is best adapted to an investigation of the development of the sporangia is not very suitable for an examination of the histology of the stem. Accordingly I have not attempted to make an exhaustive study of the latter, or of the vascular bundles of the leaf. Still the arrangement of the stem tissues is so peculiar that a few remarks will not be out of place. There is probably little variation in this respect in the different species. *I. echinospora* and *I. Engelmanni* agree very closely with *I. lacustris* as figured and described by Farmer (1), whose account is the latest and best dealing with the structure

of the vegetative organs. The center of the stem is occupied by a mass of short spiral and reticulated tracheids interspersed especially near the periphery with less numerous parenchymatous cells. The peripheral parenchyma is not sufficiently aggregated or continuous to form a xylem sheath such as occurs in the leaves. The xylem region is surrounded by a ring of tabular cells of glistening white appearance, thick-walled and empty towards the center but thin-walled towards the outside and more or less banded with incomplete layers of starch-containing cells. The cells are arranged in pretty regular radial rows, whether examined in longitudinal or transverse section. This ring is usually designated the prismatic layer, and very frequently, after Russow (1), the phloem. Russow claimed to have traced a continuity between the prismatic layer and the phloem of the leaf. I have not been able to satisfy myself of any organic continuity, but even did it exist it seems to me very questionable whether that would be a sufficient reason to justify Russow's view. No clearly defined sieve-tubes, the essential elements of the phloem, have ever been found either in the stem or in the leaf; and besides the inner cells of the prismatic zone are known to become secondarily thickened and transformed into xylem tracheids. The cells marked *o* in *fig.* 5 are in this process of transformation. Does not this indicate, if not a xylem character, at least the undifferentiated nature of the prismatic cells? A transformation of phloem into xylem would be, to say the least, an anomaly. In view of these difficulties to which may be added another — the relation to the cambium — it seems better to drop this application of the word phloem until its justification shall be established on physiological grounds. A small portion of the prismatic layer is shown in *fig.* 5.

Immediately outside the prismatic layer and indistinguishable from it except in the staining and size of the cells, is a zone of meristem which by its active division gives origin outwardly to an immense mass of cortex, and internally adds slowly to the prismatic layer. This zone is the so-called cambium. Its cells contain deeply staining plasmic contents in addition to starch

(*fig. 5*). Whether or not the dividing cylinder is more than one cell-layer in thickness I could not determine.

The cortex is very bulky and consists throughout of isodiametric parenchymatous cells abundantly filled with starch together with a little oil. The cells undergo no divisions, but as they are forced outward by the activity of the cambium they increase very greatly in size (*figs. 6* and *7*). In this way by the growth of the cells in all directions the cortex expands upwards and downwards as well as outwards, and as a result carries up the older leaves to a height considerably above the stem apex. This is illustrated in *fig. 68*, in which the unbroken lines represent the form which the cortex would take if its cells underwent no enlargement as they are pushed out from the cambium, and the dotted lines the form and dimensions which it actually assumes.

The stem apex lies at the base of the conical depression formed in the manner just explained. In small plants it is distinguishable in longitudinal sections as a slight elevation (*fig. 3*); in older plants it is merely a flattened area between the bases of the young leaves (*fig. 4*).

The method of growth of the apical meristem was first correctly described by Hegelmaier (2). Hofmeister (1) had erroneously ascribed it to the segmentation of an apical cell, having been led to that conclusion probably by too exclusive study of young plants. There is neither an apical cell nor such a group of initials as might result from the division of a rectangular apical cell like that of the Marattiaceæ. Only two or three layers of cells show their meristematic nature by their contents. The superficial layer appears to divide only in an anticlinal direction except when young leaves are about to be formed; but this layer, as Hegelmaier showed, can on no account be regarded as a dermatogen.

Although all the species of Isoetes are perennial, only a small portion of the plant persists from year to year. The roots, the leaves, and the bulky cortex are shed or decay annually, and are as often renewed from the stem apex and the meristematic zone which surrounds the small central permanent cylinder.

THE LEAF AND LIGULE.

In its earliest recognizable form the leaf rudiment seen from above is a crescent-shaped band of meristematic cells, curved about the stem apex. Sections show that it arises from the superficial cells of the stem apex, and is soon pushed up into a low broad mass, highest in the middle and inclined inwards. The ligule appears very early, and the leaf becomes distinguishable into a proximal part somewhat triangular in section and destined to bear the sporangium, and a distal part approximately circular in section and destined to become the chlorophyllous region. In correlation with the rapid development of the sporangium, the growth of the leaf is at first almost confined to the basal region. Compare, *e. g.*, the three leaves shown in *fig. 8*; tranverse sections would show the rapid growth of the basal region in a still greater degree. This region continues to widen as the leaf is pushed outward, by the formation of new leaves and the diametral enlargement of the stem; but longitudinally, except for a slight addition below the sporangium, there is only sufficient growth to accommodate the sporangium, velum, and ligule.

When the sporangium is well under way the region of rapid multiplication and growth of cells is transferred to the part above the ligule. The cells here are arranged with beautiful regularity, and growth is so rapid that this soon becomes the most prominent part of the leaf. The maximum diameter, so far as the number of cells is concerned, is speedily attained, and growth thereafter is only in the longitudinal direction. At first every part of the leaf rudiment is meristematic, but in a short time the apex passes over into permanent tissue. This change into permanent tissue progresses gradually downward until finally the whole leaf is involved. For some time a region of ever narrowing extent above the ligule continues in active division, but there is present no sharply marked or persistent meristematic zone, as seems to be implied in Farmer's account. The leaf is still quite small when all cell divisions have practically ceased, and its further elongation, which may amount to

400 or 500 per cent., is accomplished by the growth of the individual cells.

The formation of the air cavities is interesting, since it is comparable in some respects to the differentiation of trabecular and sporogenous tissue in the sporangium. In a leaf such as is represented in cross section in *fig. 9*, there is yet no sign of the air chambers. Increase of diameter is actively going on and the whole leaf is still meristematic. In the leaf shown in *fig. 10* the position of the future air chambers is indicated by four symmetrically placed groups of cells which have lost most of their contents. The peripheral cells of the leaf, the central cells, and four radiating bands which appear in cross section as spokes arranged in the form of the sign + continue to grow and are distinguishable by their larger more densely filled cells. Stained with Delafield's haematoxylin and erythrosin these cells show deep red cytoplasmic contents and large nuclei in which the red staining predominates; while in the areas which are to become air chambers the cytoplasmic contents have almost entirely disappeared, but the nuclei still retaining their chromatin stain intensely with haematoxylin. When only a nuclear stain is employed, such as iron-alum-haematoxylin, the four non-protoplasmic areas are rendered very prominent by their black nuclei. Longitudinal sections show that the regions which are thus sharply distinct in cross section run lengthwise of the leaf in unbroken bands from just above the ligule nearly to the apex, and there are as yet no air cavities.

The air chambers are formed lysigenously. The growing tissues generate a tension in the empty cells, and as a result these are ruptured irregularly, and small cavities appear, separated by diaphragms or plates of cells extending across from the central to the peripheral growing regions. As the leaf elongates, the air cavities increase in size, while the diaphragms drawn farther and farther apart lose their protoplasm to the surrounding cells. When once this splitting into diaphragms and cavities has occurred, it is not repeated; there remains no meristem in which they may be generated. Occasionally single diaphragms

of unusual thickness may be again ruptured, but no considerable increase in their number ever occurs after their first formation.

It is easy with the low power of the microscope to count the diaphragms in leaves floating upon a little water on a slide. The usual number is from fifty to seventy, and is quite as many in young leaves three fourths of an inch long as in leaves fully formed. It is instructive, too, as proving the absence of a definite meristematic zone, to count the average number of superficial cells which intervene between the diaphragms. In very young leaves this is from three to six or eight throughout the whole length, but in older leaves it is much greater, varying from twelve to twenty in the tip region to forty to sixty in the middle and basal regions, which remain longest in the meristematic condition.

The diaphragms, I think, are quite functionless, and their existence merely incidental to the manner of origin of the air chambers. They are too delicate to serve for mechanical support, which is sufficiently secured by the four longitudinal bands already described. The position of the air chambers and longitudinal bands between them in relation to the axis of the plant is always the same as that indicated in *figs. 10, 11, 44*. Near the ligule the air spaces are less regular, and instead of four of them symmetrically placed we find many irregular ones. Behind the sporangium the dorsal longitudinal band of living cells, and sometimes the two lateral ones, are well marked, but there are no large distinct air spaces. The vascular bundle of the leaf is as characteristic as that of the stem. My observations, referring chiefly to the changes of form of the bundle, were made with the view of discovering whether there is any definite relation between it and the sporangium or the ligule, and whether it presents any evidence that the leaf of Isoetes has been reduced from a more complex type. The leaf trace can first be recognized in the base of the young leaf and in the stem region below it towards the central bundle. The xylem elements are first differentiated, and consist of five or six tracheids grouped into a cylinder and surrounded by a sheath of parenchymatous cells with dense

contents. These parts can be traced later to the corresponding parts of the axial bundle. Behind the sporangium the xylem spreads out into a broad band in which the amount of xylem parenchyma is greatly increased, and the tracheids are in five or six scattered groups. Above the sporangium the xylem contracts again into a cylinder, and lies between the cornua of the ligule base. A more striking change occurs above the ligule where the xylem elements suffer an extreme diminution, there being in that region in *I. echinospora* only a single imperfect central tracheid surrounded by a sheath of parenchyma (*figs. 9, 10*). Occasionally in *I. echinospora*, and usually in *I. Englemanni*, two, sometimes three, other such groups can be traced up the leaf.

The phloem is best represented in the chlorophyll-bearing portion of the leaf. It there consists of two strap-shaped bands on the dorsal side, more or less united by their edges, so as partly to surround the xylem. In less distinct form the phloem may be traced downwards to the region of the central bundle.

The development of the ligule was accurately described by (Hofmeister 1), and also by Hegelmaier (1). The latter refers its origin to more than one cell. Since the former gives few figures, however, and the latter none, I shall again briefly outline the course of growth and illustrate it with a few drawings. The ligule originates from a single large vesicular cell protruding from the ventral face of the leaf rudiment. Provision for its rapid growth is shown in the large size of the nucleus of this cell, and the density of the cytoplasm (*figs. 12, 13*). The first division is always parallel to the face of the leaf (*figs. 14, 15*), and usually the second division is parallel to the first. The ligule of *I. lacustris* is described as passing through a filamentous stage; but in *I. echinospora* and *I. Engelmanni* it is hardly worth while to distinguish such a stage, for the filament never consists of more than three cells. The terminal cell then divides in a vertical plane at right angles to the first wall (*figs. 16, 18*). Other vertical divisions follow until the ligule has become a plate of cells of very regular arrangement. *Figs. 18*

and *19* are median sections of the ligule made tangentially to the face of the leaf. Longitudinal sections are shown in *figs. 26, 27, 28, 33, 35*. Growth in length and breadth continues very rapid, and the ligule soon overtops the leaf (*fig. 8*). For some time it remains a single layer of cells in thickness, but eventually it becomes double throughout most of its extent. The doubling begins in the middle region near the base and extends in all directions, never reaching the apex or margin however, which remain to the last but one layer in thickness (*fig. 21*). The expanded part soon reaches its maximum growth. Not so the foot region; this becomes quite massive and deeply embedded in the tissue of the leaf, especially at the sides which grow upward and downward into two prominent cornua. *Figs. 22–25* may help to explain the form of the base of the ligule. *Fig. 25* is a transverse section of the leaf cutting across the cornua above the main place of union of the ligular and leaf tissues. Sections below it show the cornua connected by a transverse band embedded in the leaf; and sections still lower would show portions of the cornua only. The other figures need no fuller explanation than that accompanying the plates.

Along with the growth of the ligule there comes about a differentiation of the cells composing it. There may be said to be four regions. The base is closely surrounded by a layer of small deeply-staining gland-like cells (*s* in *figs. 22, 38*) which we may call the sheath. It forms a conspicuous layer, everywhere investing the base of the ligule, and becoming continuous with the superficial cells of the leaf. Next to the sheath is an irregular layer or band of large empty cells, the *glossopodium* (*g* in *figs. 22, 38;* see also *figs. 23–25*). The glossopodium appears to form the base of the ligule, but the true base includes the sheath which, as a study of the development shows, is derived from the lowermost cell of the young ligule (*fig. 38*). Above the glossopodium are smaller cells containing protoplasm and forming the greater part of the ligule. The apex and margin of older ligules constitute the fourth region; the cells are

shrunken and contorted, their nuclei broken down, and the cytoplasm disorganized.

A study of the ligule of Isoetes to be complete must be accompanied by a comparative examination of the ligule of Selaginella. With this in view I have studied the origin and growth of the ligule in *S. Martensii* and *S. apus*, and compared my sections with the excellent drawings of Professor Harvey Gibson (2). Professor Farmer (1) has expressed the view that the ligules of Isoetes and Selaginella have little in common except their position and name. I have been led to quite the contrary conclusion, to hold in fact that there is a very close homology between the two. What has appealed most to me, in addition to the position of the organs, is the similarity of the regions of which both are seen to consist. The ligule of Selaginella has a glossopodium of large empty cells, sheathed by a gland-like layer, and shows also two upper regions, one of living and one of disorganizing cells. The two are alike also in the absence of chlorophyll, starch, and intercellular spaces; and both show their embryonic character by passing their maximum of growth before the leaf has reached its greatest functional activity. Differences are to be expected, of course, and are chiefly these: the ligule of Isoetes arises from a single cell, that of Selaginella from a group of cells; and, whereas the ligule of Isoetes is almost from the beginning a conspicuous part of the leaf, that of Selaginella is rather late in making its appearance, no trace of it being discoverable until after the sporangium rudiment is plainly perceptible.

THE SPORANGIUM.

The sporangium has repeatedly been made the object of investigation during the last fifty years. Hofmeister (1) was the first to make a careful study of its origin and development. Though his view that the sporangium can be traced back to a single cell has been discredited by later observers, I hope to show that his error was largely due to his exclusive dependence upon longitudinal sections. Except for his failure to see the true

nature of the sporangium rudiment as a transverse row of cells, his account is surprisingly accurate when the imperfect methods of sectioning and staining of that time are taken into consideration.

According to Hegelmaier (2) and Tschistiakoff (1) the sporogenous tissue is differentiated out of a considerable mass of deep-lying meristem between the epidermis and the vascular bundle.

Goebel (1) agrees substantially with the two preceding authors, but is more explicit in his description. The *Anlage* of the sporangium according to him is a group of cells of the leaf base, chiefly the three upper layers. The outer layer gives rise to the sporangium wall, and the hypodermal layer to the archesporium from which all the spore mother cells, trabeculæ, and tapetum are derived. Goebel's account, as confirmed and restated by Sadebeck (1) in Schenck's *Handbuch der Botanik*, has formed the basis of all the text-book descriptions of the sporangium of Isoetes written since that time.

The latest student in this field is Bower (5), whose description is confirmatory of Goebel's except that he traces the origin of the sporangium to a group of superficial cells. This difference, however, is of the very greatest importance. For whereas the derivation of the archesporium by periclinal divisions of superficial cells is the rule in Pteridophytes, the origin of the sporogenous tissue from a hypodermal layer separated from the beginning from the epidermis is a spermatophyte character. The result of Bower's work then is to put Isoetes in line with other Pteridophytes in respect to the origin of the archesporium.

My own results are in the main confirmatory of Bower's as to the origin of the sporangium, though with variations in minor details which may be due to specific differences (Bower studied *I. lacustris*); but as to the later stages of development, especially of the megasporangium, I cannot make my observations harmonize with any accounts hitherto written.

It will, of course, be apparent, when so many discrepancies appear in the descriptions of different investigators, that the

study must be one which involves considerable technical difficulty. This is attributable (1) to the absence of an elongated axis and internodes and the consequent crowding of the sporophylls, and (2) to the early appearance of the sporangium and the consequent difficulty of distinguishing it from the other meristematic tissues in which it is placed. The kinds of evidence on which I have relied in my interpretations may be stated briefly as follows:

1. Study was made of sporangia whose sporogenous tissue was already distinct and unmistakable. Then by comparisons with successively younger sporophylls the attempt was made to trace the sporangium to its earliest rudiment.

2. A careful comparison was made of sections in the three planes, longitudinal, transverse, and tangential. This involved the waste of a great deal of material. For it will be made clear by a glance at *fig. 4* that sections made longitudinal to the stem could give longitudinal sections of very few young leaves, and oftener than not would fail in this altogether, since the leaves have a spiral arrangement; while, in order to obtain transverse and tangential sections, one must cut obliquely to the stem without possessing any clue by which to determine the proper angle of obliquity.

3. The position of the vascular bundle enables one to determine whether the sections are truly longitudinal, and which of a number of serial longitudinal sections is exactly median. This help is available only after the sporangium is distinctly outlined, and somewhat advanced in development, for in case of very early stages of the sporangium, the vascular bundle has not yet been differentiated.

4. In such early stages one must depend very largely upon the ligule, which in position and outline is so definite, and in manner of growth so regular as to make it of the highest importance in assisting one to orient the sections.

5. The sporogenous tissue is often distinguishable from vegetative tissue by a difference in staining. There are three periods when this difference is most manifest. The superficial

cells which form the earliest rudiment of the sporangium frequently take a distinctive cytoplasmic staining, especially in material fixed in Flemming's solution. It must be confessed that this means of recognizing sporogenous tissue is not so trustworthy as one could wish, for at this period of the leaf's history all the tissues are meristematic, and hence readily susceptible to protoplasmic stains. One who studies the origin of sporangia in Lycopodium or Selaginella meets with the same difficulty in those plants, a difficulty in my experience quite as great in these cases as in Isoetes. When the superficial layer of the sporangium has assumed its character as an epidermis, the deeper lying sporogenous cells are easily distinguishable by stain reactions from the surrounding tissues. At a later period the spore mother cells selected out of the general internal mass of the sporangium become quite distinct on account of their denser contents and more intense staining.

Longitudinal sections of young leaves show no space between the base of the ligule and the stem. At this time there is still an active uplifting of cells above the general stem level, a continuance of the process by which the leaf first emerged. When the ligule has grown sufficiently to contain eight or ten cells in longitudinal section the space below it is occupied by one large cell with dense cytoplasmic contents (*fig. 26*). The next change which takes place is a transverse division of this cell as shown in *figs. 27, 28*. Comparisons of successive serial sections show that the two cells shaded in *fig. 27* form the middle of a group of cells arranged transversely to the leaf. This group of cells, distinguishable in good preparations by their deeper staining and larger nuclei, constitute the rudiment of the sporangium. In order to learn its extent and arrangement recourse must be had to transverse and tangential sections.

Most transverse sections of this early stage of the sporangium show that it is five cells in width. Whether or not these can be traced back to a still smaller number I am in doubt. *Fig. 29* certainly shows an example where the transverse row consists of only three cells, and it is clear that the shaded cells of *fig. 30*

may have had their origin in three similar to those of *fig. 29*. But I have succeeded in getting only two such cases as that of *fig. 29*, one in *I. echinospora* and one in *I. Engelmanni*, and have failed altogether to obtain a tangential view.

Tangential sections of the leaf at this early stage are almost uninterpretable. The face of the leaf is so closely pressed against the back of the next younger one that it is quite impossible in most instances to distinguish the tissues of the two leaves or to determine what is a truly tangential section. That shown in *fig. 31* was such as to admit of certain interpretation. The shaded cells occupy the surface of the leaf and clearly correspond to the group which we have already examined in longitudinal and transverse sections. It is probable that another cell seen in the adjacent section to the left of those figured belongs to the same group, making the total number of cells seven.

It is evident from a comparison of my *figs. 26–28* with *figs. 104–106* of Professor Bower's plates, that the longitudinal growth of the leaf base of *I. lacustris* is much more rapid than that of *I. echinospora;* and his figures though not his text suggest that the six superficial cells which make up the sporangium *Anlage* are derived from not more than three rows and probably from but two. If this suggestion be correct, it would bring Bower's and Hofmeister's accounts, so far as regards longitudinal sections, into harmony with each other, and with the foregoing account of *I. echinospora.*

The young sporangium, situated as it is on the hollow side of the leaf crescent, projects little if at all from the surface. By its rapid growth, however, it soon forms an oval prominence at first wider than long, then nearly circular in surface view, and finally considerably longer than wide. In its development I have not been able to establish any regular order of sequence. Starting from such a beginning as figured in *fig. 26*, it is certain that transverse and longitudinal divisions are the first to occur. Then periclinal walls appear (*fig. 30*). The middle cells of the sporangium rudiment are at first most active in dividing, not only in respect to surface growth, but in periclinal divisions also.

Sections adjacent to that represented in *fig. 31* show three or four hypodermal cells which have been cut off from the middle cells of the group and evidently belong to the same series.

There is at no time a single complete hypodermal layer which may properly be termed an archesporium. For when the middle cells have just completed their periclinal divisions the lateral cells are still undivided, and by the time the lateral cells have undergone their first periclinal division the middle of the sporangium is at least three layers deep. A very good example of this is seen in *fig. 42*, which represents the side of quite a large sporangium.

The growth of the sporangium is carried on most actively by the two or three outer layers of cells, as is evidenced by their large size and deeper staining, and the frequency with which they are found in karyokinesis. The divisions of the superficial layer are by no means limited to those in anticlinal planes, as is usually the case with the external cells of sporangia, but for a long time they continue to add to the inner mass by periclinal divisions. In the sporangium of which *fig. 39* shows a section, as many as eight or ten of the external cells were in the act of periclinal division. Even in so old a sporangium as that shown in *fig. 43* the same process is still in continuance. The cells marked with a cross have evidently been derived from the external layer. Though in older sporangia the additions so made go to form part of the sporangium wall, there can be no question that in the younger sporangia they add to the true sporogenous tissue. The bearing of this fact upon the question of what constitutes an archesporium will be considered further on.

It seems necessary to digress at this point in order to make clear some features in which the preceding account differs from what has been recorded by previous observers. Both Hegelmaier (2) and Tschistiokoff (1) assert that the wall of the sporangium is from the beginning ("von Anfang an gesondert") separated from the inner complex, and emphasize with great distinctness the deep-seated origin of the sporogenous tissue.

Goebel (1) states his approval of Hegelmaier's view, but the occasional periclinal division of the external cells does not escape his notice, though he considers it as merely adding to the thickness of the wall. Bower (5), on the other hand, observed both the superficial origin of the sporangium and the failure of the first periclinal divisions to completely delimit the archesporium.

As already stated, I do not find the outer wall separate from the sporogenous complex from the beginning. On the contrary, it is distinctly active in increasing the dimensions of the sporangium. Ultimately the superficial layer loses some of its protoplasmic contents, and assumes the appearance of an epidermis. It sometimes happens that this separation of a wall layer occurs quite early (*fig.* 41), but oftener it is not till the sporangium has come to consist of many hundred cells. Even then periclinal divisions do not entirely cease.

According to my observations there is no regularity in the arrangement of the cells within the sporangium. The discovery of this was a great surprise to me, for Goebel's statement is very explicit: "Each of the cells composing the archesporium has an independent growth," and in this he has been corroborated by Sadebeck and Farmer. Bower has not traced the history of the sporangium with any fullness; he merely states that his results are confirmatory of Goebel's and his figures certainly convey the impression that each cell of the archesporium has an independent growth. But he has made use of the same style of drawing in representing the sporangia of other genera (Lycopodium, Selaginella, Equisetum), in which no such claim is made. In view of my own observations and of Bower's drawings, it is difficult to know just how much is meant by the phrase "independent growth."

In the case of bryophyte antheridia the primary spermatogenous cells are clearly distinguishable throughout the whole development of the antheridium, although each may become divided up into a hundred or more sperm mother cells. The individuality of the original cells is marked in several ways:

their outer walls remain straight and become thicker than those which subsequently appear within them; and the incomplete separation of the derivatives of any single primary sperm cell from one another and their complete separation from those of other primary cells are shown by their dividing concurrently. I have frequently observed in the antheridia of Polytrichum, Porella, Marchantia, and Asterella that all the cells derived from one of the primary sperm cells enter into karyokinesis together, finish their division, and enter into the resting condition together, quite independently of what may be going on in the derivatives of other primary cells. In such cases it is quite proper to speak of an independent growth; for the separation and isolation of each group by thickened walls are sufficient to insure a simultaneous exposure and obedience of all the cells to the physiological stimulus which induces karyokinesis.

Are there any indications of such independent growth in the sporangium of Isoetes? I can find none, either in the arrangement of the tissues or in the presence of thickened walls which mark the boundaries of the original archesporial cells, or in the simultaneous entrance of the cells of each group into the phases of division. All the mature cell walls of a growing sporangium are of equal thickness; and in marked contrast to what is seen in the leaves there is no regularity of stratification or lining-up of the cells. I am forced to conclude that the sporangium of Isoetes (at least of *I. echinospora* and *I. Engelmanni*), just as the microsporangium of angiosperms, grows as a unit and not as a number of individual segments.

Before continuing the subject of the development of the sporangium it will be convenient to consider the formation of the velum. The velum makes its appearance very early in the history of the sporangium, almost as soon in fact as the first periclinal divisions of the superficial cells. It is formed immediately below the ligule. Hofmeister (1) says: "Of the two cells into which by a transverse septum the cell underneath the place of insertion of the ligule is divided the upper one becomes the primary cell of the velum and the lower the primary mother cell

of the sporangium." It has already been said that Hofmeister was in error because of failure to notice the lateral extension of the sporangium rudiment. Even allowing for this, however, I am not able to agree fully with his account. It appears rather that the upper tier of cells while giving rise to the velum makes some additions at the same time to the sporangium. In other words, the velum is a sterilized portion of the sporangium. Some sections seem to admit of this interpretation only, though others, such as *fig. 32*, are not unfavorable to the view that the separation of velum and sporangium proper is accomplished by the first transverse division of the sporangium rudiment.

Early stages of the velum may be seen in *figs. 32–36;* it is at this time a transverse row of slightly projecting cells. Its cells soon become comparatively empty, contrasting strongly with the young sporangium. Growth is very rapid and in an upward oblique direction; in some cases there is a tendency to a downward growth also, such as obtains among terrestrial species.

The velum reaches its full size much sooner than the sporangium, and is not affected by the changes which determine the character of the latter. The cells of the interior become large and lose their contents; those of the inner surface layer—that adjacent to the sporangium—are smaller and more regular in size and outline, and have a semi-glandular appearance. In many species of Isoetes many of the cell walls of the velum and of the leaf region adjoining the ligule become lignified and take on spiral and annular thickenings. *I. echinospora* and *I. Engelmanni* offer no exception in this respect, the thickenings being much more pronounced in the latter species. The change first appears in proximity to the ligule, and spreads thence into the remoter parts of the velum and of the leaf. The thickened cells never have any connection with the vascular bundle (*figs. 23, 24*).

FURTHER DEVELOPMENT OF THE MICROSPORANGIUM.

In origin the two kinds of sporangia are identical, and for a considerable period of their development they exhibit no

observable difference. The general statement of the text-books, following Goebel and Sadebeck, is that they follow the same course of development only so far as the formation of the archesporium, and thereafter may be distinguished by their manner of growth. It is said that in the megasporangium certain archesporial cells divide only by periclinal walls, but in the microsporangium all the archesporial cells divide both anticlinally and periclinally, and that in this respect the two are distinguishable from the archesporial stage on. Such is not the case in the forms which I have studied. In these all the archesporial cells, whether of megasporangium or microsporangium, undergo divisions in all directions, and the similarity of the two kinds of sporangia continues much beyond the archesporial stage. Not only do they agree in origin, but up to a time when they are eight or ten cells deep, they agree absolutely in manner of growth, and exhibit no histological features by which one may determine whether a given sporangium will bear microspores or megaspores.

As an example, consider the sporangium of which *fig. 43* represents a section. It had advanced so far beyond the archesporial stage as to contain about 8000 cells. From the position of its sporophyll we may infer it was destined to become a megasporangium. But there is nothing in the arrangement or character of the cells or in their mode of division to warrant that prediction, or to enable us to say such a group of cells will become a trabecula, and such a group will produce spores. It has the characters neither of a megasporangium nor of a microsporangium, but is as yet quite undifferentiated.

The first changes which occur to mark the microsporangium are those which lead to the differentiation of the spore mother cells from the trabeculæ, sporangium wall, and tapetum. Previously there has been no essential difference in the cells as to size, form, or contents, excepting the external layer. But when the sporangium is approaching a limit of cell multiplication, that is, when the number of cells is 15,000–20,000, certain regions begin to lose their power of division and reaction to stains, while

other regions become more active in division and more deeply stainable. The former may be called the sterile regions, since they form the walls, trabeculæ, and tapetum, and the latter the fertile region, since they give rise to the spores. Even in unstained sections the difference is noticeable as one of relative abundance of protoplasmic contents.

At first it is difficult to see clearly the limits of the regions or to make out their arrangement. But in older sporangia they are seen to be disposed in irregular bands extending from the base of the sporangium outwards to the wall. The published drawings, and unfortunately in some cases the written description also, are calculated to convey an erroneous idea of the trabeculæ. They are not partitions, but, though irregular in outline and frequently branched and anastomosed, are comparable rather to pillars. It is accordingly incorrect to speak of the sporangium as chambered, for the fertile cells are not segregated into loculi, but form a continuous mass pierced here and there by the trabeculæ. It is hoped that *figs. 44–47* will make the relations of the trabeculæ clear. The shaded portions of these drawings represent the fertile regions, and the unshaded portions the trabeculæ and walls. The continuity of the sporogenous mass is clearly seen in the tangential section (*fig. 46*).

A more detailed account of the development of the microsporangium will now be given. *Fig. 48* shows a small portion of a microsporangium in which the differentiation into sterile and fertile regions has just begun. The fertile cells stain deeply and are still rapidly multiplying, as is evidenced by the many karyokinetic figures. The sterile cells have almost entirely ceased divisions, though here and there a dividing cell may be found. It is important to notice that the one character in which the two regions differ is in the relative abundance of protoplasm, the fertile cells being densely filled with deeply staining cytoplasm, while the cytoplasm of the sterile cells is beautifully vacuolated. In all other respects the cells of the two regions are essentially alike. They are not markedly different in size,

or in the size and appearance of their nuclei, nor is there anything in their arrangement to suggest a difference in their origin or growth. In fact, as Professor Bower has pointed out, there is here a most excellent illustration of the sterilization of sporogenous tissue.

The trabeculæ at this age show about 15–25 cells in cross-section (tangential section of the sporangium), and are more or less cylindrical. There is as yet no tapetum. Towards the outer and inner sides of the sporangium the trabeculæ are continuous with about three layers of cells which form the sporangium wall (*fig. 49*). That the trabeculæ and walls are of the same nature, both being the result of sterilization of potentially sporogenous tissue, is proved not only by the similarity of their cells, and their passing uninterruptedly into one another, but also by their relation to the tapetum, which is formed out of the layer that lies next to the spore mother cells.

The inner cells of the trabeculæ, those which become the trabeculæ proper (*i. e.*, exclusive of the tapetum), are at first isodiametric and in no way different from the outer ones. But while the latter are undergoing a transformation into tapetum, the former undergo changes which are dependent on the growth of the sporangium. As the dimensions of the sporangium increase — a change which goes on rapidly at the period when the sporogenous cells are multiplying — the trabeculæ are necessarily lengthened. This is accomplished, not by division of the cells, but merely by their elongation. At the same time they suffer a lateral compression from the growing sporogenous cells and become flattened (*fig. 50*). The tabular form of the cells doubtless furnishes the ground for the common view, which ascribes the form of the cells to the direction of their division planes. Such a view is incorrect, however, for divisions have entirely ceased in this region before the elongated form of the cells is attained. The shape of the cells is easily accounted for by their growth in the one direction possible for them while yielding to the pressure of the turgescent mother cells.

In this connection it may be remarked that with the possible exception of the tapetum all the cells of the sporangium, after

losing their power of division, enter upon a period of growth which is quite comparable to that occurring in vegetative meristems. The difference in size of the sporangia represented by *figs. 43* and *63*, which are drawn under the same magnification, is due partly, it is true, to increase of the number of cells, but a glance at the two figures shows there has been also a decided growth of the individual cells.

Accompanying the modification of the trabecular cells, there is a change of form of their nuclei. These become first elongated and oval (*fig. 50*), and finally spindle-shaped, suggestive of the changes which attend the development of the vascular strand out of the tissues of a growing point. Instances of much greater elongation than that shown in *fig. 51* are frequently met with, though in other cases the changes are comparatively slight. The nuclei at this time are relatively large and prominent, and appear to form the center of aggregation of what little cytoplasm still remains in the trabecular cells. In old sporangia the cells of the trabeculæ are nearly or quite empty, and much compressed.

Bower has discussed the function of the trabeculæ. They may serve for mechanical support of the sporangium, or to afford a larger nutritive surface, or, since the two functions are not incompatible, for both. The relation of the trabeculæ to the base of the sporangium where it is closest to the vascular bundle, and the resemblance of the nuclei to those of plerome regions in general, suggested to me that the trabeculæ might be the channels through which nutriment is supplied to the spores; but the suggestion is not borne out by observation. It is clear that in a hydrophytic plant no elaborate apparatus is needed to provide the sporangium with water, which can easily enter directly from the outside; and an examination of my sections shows that the organized food stuffs, such as starch and oil, pass to the spores through the inner wall of the sporangium, and not through the trabeculæ.

The tapetum, as already stated, is organized out of that layer of the sterile cells, whether of wall or trabeculæ, which is in contact with the fertile cells. At a stage between those shown in *figs. 48*

and *50*, the cells of this layer multiply rapidly. They are frequently found in mitotic division, with the axis of the spindle always perpendicular to the surface of the trabeculæ or sporangium wall. Divisions may still go on here after the spore mother cells have reached maturity, and the changes of the trabeculæ are nearly complete. In this way the tapetal cells become very numerous, but reduced in size. They form but a single layer except in limited areas, where a doubling may sometimes occur.

At first the tapetum is not deeply stained (*fig. 50*), but as the spore mother cells prepare for their tetrad division, the tapetal contents increase in density, and they continue to do so until they surpass young spores in this respect.

From what has been said, and from *figs. 47*, etc., it will be understood that the tapetum completely invests the trabeculæ and sporangium wall, forming a lining layer everywhere between the spore mother cells and the sterile regions. It is a persistent layer, and in this respect is to be contrasted with that of most ferns and angiosperms. In these latter the walls of the tapetum break down and are dissolved, the cells become disorganized, and their materials, mingling with the other contents of the sporangium, are used to nourish the mother cells or young spores. In Isoetes, however, as in Lycopodium and Selaginella, no such disorganization of the tapetum occurs. Its cells do not fall apart and its walls are not absorbed. In old sporangia it is still recognizable, though often its contents have been lost and the walls are pushed nearly together.

Probably the tapetum can best be regarded as a gland or layer of glandular cells. If so, the manner of action in a persistent tapetum, such as that of Isoetes, Lycopodium, and Selaginella, must be quite different from what it is in a tapetum which is regularly disorganized and absorbed. In the one case the nutrient substances secreted by the cells must be passed on through the walls into the cavity in which the young cells are growing. In the other case there can be little or no passing of nutrient substances through the walls, but at the proper time the

secreted materials are rendered available by the total collapse of the cells.

In many plants also, especially in those in which the tapetum undergoes complete disorganization, it is common for the tapetal cells to become multinucleate, the division of the nuclei being sometimes accomplished by karyokinesis, but mostly by amitosis. The cells of the tapetum of Isoetes, in this respect again agreeing with Lycopodium and Selaginella, are uniformly uninucleate.

In almost every sporangium examined the number of layers of cells outside the fertile regions when they first become distinct is three. In a very few cases there were four layers. As already shown, the innermost of these becomes tapetum. Of the other two layers, one, apparently the hypodermal, usually undergoes division, so that the wall region ultimately consists of three layers outside the tapetum.

At the base of the sporangium, between it and the vascular bundle, are a few layers of cells which may be regarded as the inner wall of the sporangium. The exact origin of these I have not been able to make out. Whether, like the outer wall, they are derived from the sterilization of sporogenous tissue, or whether they are derived from the tissues underlying the original archesporium, I cannot say. It is always difficult in all sporangia except the very youngest to define the exact inner limits. Between the vascular bundle and the three or four outer layers where growth and division are most actively carried on, there is a mass of small cells staining deeply. Such a section as *fig. 38* makes it probable that all the tissues between the parenchymatous sheath of the xylem and the outside arises from the sporangium *Anlage*, and that therefore the inner wall arises also by sterilization.

The formation of the microspores in Isoetes takes place in much the same way as in other vascular plants. After the fertile regions have ceased their cell divisions, the cells and their nuclei pass through a period of rest and enlargement. The nuclei especially increase in size and become rich in chromatin. At the same time the cytoplasm remains dense and never

shows the vacuolated appearance of the sterile cells. Shortly afterwards the mother cells break away from the tapetum, which from this time on gains in density and apparent activity. The mother cells, at first in a continuous mass, soon break up into smaller and smaller groups of cells by the enlargement of the cavity in which they float. Finally the individual cells fall apart and round up, and pass rapidly through the two divisions by which the microspores are formed.

No attempt was made to follow closely the cytology of these divisions because it was found impossible to make any satisfactory observations on the corresponding divisions of the megaspore mother cells. The following notes may however be of interest. The achromatic figures appear to have a polycentric origin, and the chromatin passes through a synapsis stage. All the nuclei make their preparation for division and begin to divide almost simultaneously, and this notwithstanding their immense number. It is possible to find a better series of karyokinetic figures in a single sporangium of many ferns, where there are but sixteen mother cells, than in an Isoetes microsporangium where the mother cells number three or four times as many thousand. This I think may be regarded as an additional proof of the growth of the sporangium as a unit, and not as an aggregation of segments.

In the majority of cases the two divisions are of the type which is characteristic of cycads and monocotyledons, and has been called "successive;" that is, the first division of the nucleus is followed by the formation of a cell wall before the immediately following division of the daughter nuclei (*fig. 53*). The spores in this case are bilateral and may have their nuclei in one plane or in two planes at right angles to each other. But it is not at all infrequent to find the divisions of the simultaneous type; that is, the first division of the nucleus is not attended by cell division, but before a wall is formed between the daughter cells each new nucleus begins its second division (*fig. 54*). In this case the spores may be of the bilateral type, as in *fig. 55 a* and *b*, or they may be tetrahedral as in *fig. 55 c*. Much

diversity may be found within a single sporangium. *Figs. 53 a, b, c,* and *54 a, b, c,* were all taken from the same section of the same sporangium. Probably the variation in this respect is not of great importance except as indicating that the divisions of Isoetes have not acquired so definite and settled a character as those of most other plants.

Although the nuclei of the young spores may arrange themselves in typical tetrahedral fashion, there is an important difference between their relation here and in the tetrahedral divisions of dicotyledons, Lycopodium, etc. In these it is well known that all four nuclei (of such a stage as *fig. 54*) become connected by spindle fibers, and that the walls separating the spores are formed in connection with the thickening of the cell plates of the six spindles. In spite of careful search I have been unable to find in Isoetes any such sextuple spindles. The daughter nuclei are connected only in pairs, as in *fig. 53* or *54*. In what way the spore walls originate in such cases I cannot conjecture. It seems certain they are not formed in connection with the achromatic figures, unless it is possible that the cell plate, which is always present in the first division, may make its influence felt later on, and ultimately serve as the basis of the wall.

The young tetrads soon fall apart, and the individual spores lose their angularity and round up, still retaining traces, however, of the bilateral shape impressed upon them by their manner of origin. When once the permanent form is assumed there is little further increase of size. The mature spores of *fig. 56* are little larger, it will be seen, than the newly formed spores of *fig. 51*.

An interesting phenomenon in connection with the microspores is the extreme smallness of their nuclei in comparison with those of the mother cells. One would naturally expect the relative volumes to be about 1 : 4, or the relative diameters to be about 3 : 5 (since $\sqrt[3]{\frac{1}{4}} = \frac{3}{5}$ nearly). But the volume of the microspore nucleus is really no more than one twelfth of this estimate; or to express the comparison in another way, it would need the nuclei of fifty microspores combined to equal the

volume of one mother cell nucleus. Very likely similar reductions in the volume of the microspore nuclei occur during the tetrad division of other plants, but I have not seen any other case where the disparity of size is so great, nor do I remember to have read any record of such a reduction.

The number of spores formed within a microsporangium is enormous—much greater than in any other living plant. In some species it is said to exceed a million. But the largest number I have found in *I. echinospora* is 300,000. My estimates place the average number from 150,000 to 250,000.

As is well known, no provision is made for the dehiscence of the sporangium wall. The spores are set free only by the decay of the tissues enclosing them.

FURTHER DEVELOPMENT OF THE MEGASPORANGIUM.

My observations on the development of the megasporangium differ very much from those of previous investigators, so very much, indeed, that I would be loath to present them at all had I not confirmed them again and again by long and careful study. These differences are concerned not only with the origin of the archesporium and early growth of the sporangium, which have been already spoken of, but they involve also the manner of selection of the mother cells and the origin and behavior of the tapetum. A discussion of the points at issue will be reserved until the general history of the megasporangium has been considered.

One of the first megasporangia which I sectioned presented the appearance shown diagrammatically in *fig. 67*. The two large cells *M* and *M* are evidently megaspore mother cells, but what is the group of cells *a*, corresponding to them in outline and position? It consists of six cells in all, three in the section under examination, and three others in the adjacent section. A little search discovered other similar groups of a variable number of cells, sometimes but two or three, often five or six. If the number had been constantly four the groups might have been regarded as spores resulting from a precocious division of

the mother cells. But that explanation being precluded it became necessary to determine their relation to the single large mother cells, and to learn their later and earlier history. In attempting to do so I have become convinced that a very large number of cells are potentially megaspore mother cells, that a considerable number of these make a start to differentiate themselves fully from the sterile cells, but that comparatively few are finally successful in reaching the large size and well-nourished condition necessary for the production of megaspores.

The changes which first distinguish the megasporangium occur relatively earlier than those which mark the microsporangium. In the latter, as we have seen, the first change is the separation of certain sterile regions from the fertile cells as indicated by a difference in cell contents. In the former, however, changes occur at a considerable time before there is any possibility of distinguishing the trabeculæ. When the megasporangium has reached a stage of development considerably more advanced than that shown in *fig. 43*, a change is discernible in many of the cells which form the third and fourth layers approximately. The whole sporangium has at this time entered upon the period of enlargement due to the growth of the individual cells. But in *fig. 63* it is clear that certain cells have greatly outgrown their fellows. Their well-nourished condition is attested by the density of their cytoplasm and their large nuclei, which contain many nucleoli. All these enlarged cells are engaged in the struggle to become mother cells. Which and how many will be successful will probably depend upon their holding an advantageous position with respect to the supply of nutriment, perhaps also to their having obtained an earlier start.

It does not always happen that a considerable group of cells enlarge together. Indeed, it is a comparatively rare case when all the cells of the third and fourth layers enlarge to any considerable extent. Sometimes the enlarging cells are in more or less isolated groups separated by cells of ordinary size. *Fig. 64* shows such a group of cells, taken from the side of a sporangium.

Quite often, too, it happens that one cell gets the advantage almost from the beginning. But it may be stated as the rule that there is a selection and partial enlargement of many more cells than can ultimately become mother cells, and these enlarging cells belong mostly to the third and fourth layers of the sporangium, either extending continuously across the sporangium or occurring in groups separated by ordinary cells. That this condition is associated with the selection of megaspore mother cells is proved, I think, by the fact that enlarging cells, comparable to those of *fig. 63*, are never found in the sporangia formed late in the season, that is, in those which are to bear microspores.

What becomes of the defeated cells? This is a difficult question to answer, for since there is so much variation in the early condition of the megasporangium it is impossible when examining one of the later stages to tell just what the antecedent conditions in that sporangium may have been. From the frequency with which karyokinetic figures appear in the cells surrounding the nearly mature megaspore mother cells, it seems pretty certain that the cells which have been left behind in the struggle simply divide until their products have the general size and appearance of the other cells of the sporangium. If the enlargement has not gone very far the cells retain their angular configuration; if it has gone further the cells may round up while exerting a considerable pressure on those adjacent. So I interpret the group *c* in *fig. 67*.

Fig. 66 will furnish a good illustration of the behavior of the unsuccessful mother cells, although no single section can be so convincing as a series of them. The tissues are somewhat contracted, but this defect does not hide the rounded form of certain groups of cells, and their marked resemblance, except in being multicellular, to the mother cells. The section contains but one fertile mother cell, the one labeled *m*. One other is situated in the opposite end of the sporangium, just beyond the limit of the figure. The cell *a* is undergoing division, the mitotic figure being seen in the adjacent section. An interesting fact

which goes far to explain the division of the groups *b*, *b*, is the occurrence in adjacent sections of larger undivided cells (fertile mother cells), similar to *m*, and so situated as to be almost or quite in contact with the dividing groups. Their proximity accounts for the failure of the groups *b*, *b*, to produce spores. Some of the smaller and less rounded groups probably represent mother cells which suffered an early defeat, while the larger groups represent those which held out almost to the last. Such cases as these, which can be easily duplicated in rapidly growing sporangia of the right age, are conclusive, it seems to me, when considered in conjunction with the manner of growth of the sporangium, to show that the fertile mother cells are selected by their advantageous environment and not by any strict morphological position.

The fertile mother cells increase enormously in size before dividing into spores. Their nuclei maintain a proportionate growth, and their cytoplasm remains dense though not homogeneous, and frequently contains grains of starchy matter and drops of oil.

Notwithstanding the large size of the mother cells and of their nuclei I was unable to make any detailed study of their division. About the time when division occurs, the cells seem to be peculiarly liable to suffer plasmolysis, for under the action of the fixing agent they are contracted to a mere fraction of their proper volume. When sectioned in this condition they are seen to lie free in large cavities which presumably they filled completely when living, and they stain so intensely that it is impossible to make out any details of the karyokinetic process. I have not once had the good fortune to see karyokinesis in an uncontracted megaspore mother cell, although the corresponding phase of the microsporangium offers no technical obstructions to cytological study. The liability of the megaspore mother cells to suffer contraction in the process of fixation was noticed by Kienitz-Gerloff (1) and other investigators; it is possibly associated with the entrance of the nuclei into the synapsis stage.

The young megaspores almost invariably have the tetrahedral arrangement, as in *fig. 59*. Occasionally the bilateral arrangement is found, in which case the divisions so far as observed are successive (*figs. 60, 61*).

The further growth of the megaspores, the manner in which their walls are laid down, and the storing of reserve material, were not investigated.

The arrangement and subsequent development of the trabeculæ and tapetum of the megasporangium offer, as is to be expected, a rather close homology to what is seen in the microsporangium. The trabeculæ are formed out of the same kind of cells as compose all the other parts of the young sporangium. I do not discover any grounds for considering them the product of a peculiar kind of growth. They are altogether unrecognizable in the young sporangium, and their position when first outlined seems to be determined by that of the mother cells. Not until these have been selected and considerably enlarged is it possible to distinguish the trabeculæ, which then appear as feebly-staining bands extending from front to back across the sporangium midway between the fertile cells.

The cells of the trabeculæ proper undergo the same process of elongation and flattening, attended by elongation of their nuclei, that has been described as occurring in the microsporangium. The only noticeable difference is that in the megasporangium the trabeculæ are relatively fewer in number and more massive. For example, in one case, an exceptional one, I counted 400 cells in a cross section of a trabecula, whereas in a microsporangium the number of cells in a cross section of a trabecula rarely exceed fifty, and is oftener under twenty-five. This is only another way of saying that the process of steriliza tion has gone much further in the megasporangium than in the microsporangium. The total mass of the megaspore mother cell in a sporangium is only a small fraction of that of the combined microspore mother cells, though doubtless the total volume of the mature spores in the two cases is about equal.

The tapetum is formed in this case also out of those layers of the sterile cells which border upon the fertile cells. No doubt a considerable part of it is derived from the unsuccessful mother cells; but as these are the homologues of the trabecular cells of the microsporangium, being merely sterile sporogenous cells, the homology of tapetum and trabeculæ in the two sporangia is complete. The only difference which it is necessary to notice is the greater abundance of the tapetum in the megasporangium. Instead of being a single layer it is several layers in thickness (*figs. 57, 58*), and often projects into the sporangial cavity in the form of irregular papillæ, especially from the base of the sporangium. A rounding up of the cells immediately about the megaspore mother cells, such as is described and figured by Goebel, I was never able to find.

Though the megaspore mother cells do not lie in contact with one another as the microspore mother cells do, but are isolated in groups of one or sometimes two, the cavities in which they lie become continuous in the older sporangia. This is brought about by a very great enlargement of the cavities after the formation of the spores. The enlargement seems to be due to turgescence, induced probably by the osmotic activity of the substances surrounding the spores. It cannot be accounted for by mere growth of the wall cells, nor by that of the young spores, for these do not completely fill the cavities. I have computed the enlargement of the megasporangium after all cell divisions have ceased to amount to an increase of three or four times in volume. A similar change of size, though less in extent, occurs in the microsporangium.

If the preceding account of the development of the sporangia, especially of the megasporangia, be compared with the account given by Goebel (1) and Sadebeck (1), it will be seen that the differences are considerable, and of much theoretical importance. According to these writers certain cells of the archesporium divide only by the periclinal walls which serve to cut off the primary tapetal cells. In these no anticlinal divisions occur. One cell of each of the rows formed in this manner,

apparently the innermost one, though that point is not made clear in the descriptions, becomes the megaspore mother cell.[1] In certain other archesporial cells divisions take place in all planes, but more particularly in the anticlinal direction. The products of these latter cells give rise to the trabeculæ. Vines in his text-book gives nearly the same description, but says that the archesporial cell from which the megaspore mother cell arises undergoes but a single division.

If the assertion be correct that certain archesporial cells develop only into trabeculæ and certain others only into mother cells and tapetum, it is clear that there must be two categories of archesporial cells, one set destined to become sterile, the other to become fertile; and these, although indistinguishable in appearance and size, are quite unlike in their mode of division and growth and in the ultimate fate of their derivatives. It is impossible, too, to escape the inference that the megaspore mother cells are already determined in position and number when the sporangium has got no further in its development than to the differentiation of an archesporium. Further, the sporangium must be regarded as compound, each fertile archesporial cell representing a separate sporangium, and each sterile one an imperfect wall. These conclusions, which I think are logical and necessary deductions from Goebel's description, are all inconsistent with the development of the sporangium as I have found it in *I. echinospora.*

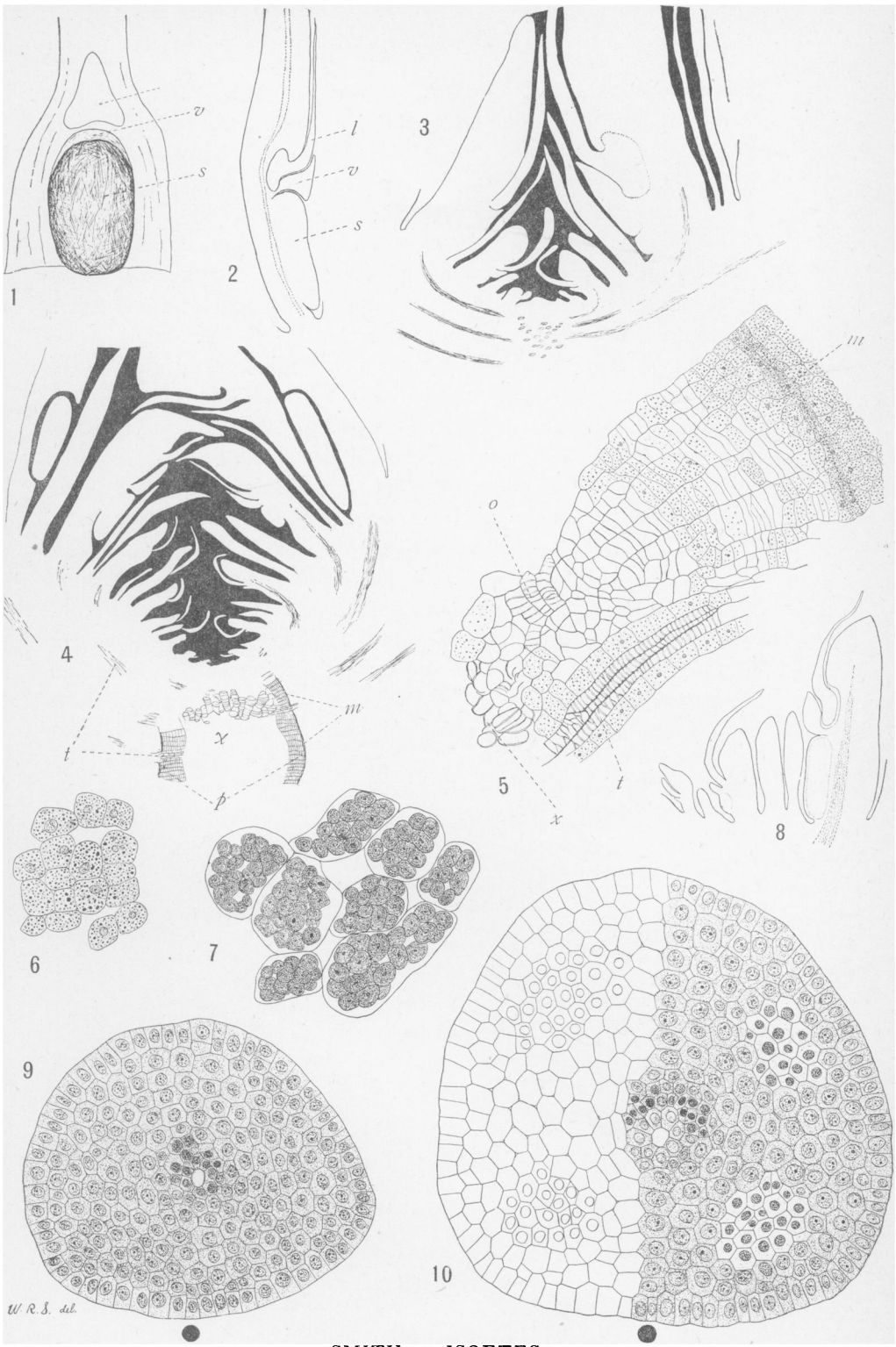
In order to bring out the points of contrast more clearly I will summarize them. I do not find any difference among the archesporial cells either in manner of development or of growth. I find no flattened tapetal cells overlying the megaspore mother cells. I find no grounds whatever for the assertion that each archesporial cell follows an independent growth, or that each megaspore mother cell represents one archesporial cell. I do not even find a single definite hypodermal archesporium which can stand as the starting point of the inferences above enumerated. On the other hand, I find the derivatives of all

[1] See, however, SCHENCK's Handbuch 3 : 392.

the archesporial cells dividing in various planes, and blending indistinguishably. The sporangium is single, not multiple, and the megaspore mother cells are not morphologically predetermined but are physiologically selected from among a large number of potentially sporogenous cells.

Though the certainty of the matter must depend upon observation, it may be pointed out that the number of megaspores has a bearing upon the question. A megasporangium contains from 150 to 250 megaspores. If we take 200 as the average, it represents fifty mother cells, that is, according to the current view, fifty archesporial cells. To this we must add at least fifty others for the trabeculæ, giving a total of one hundred archesporial cells. It does not need a very careful examination of *I. echinospora* to demonstrate the impossibility of there being so large an archesporium, for when the sporangium has a superficies of one hundred cells it is far past the archesporial stage. It is, I think, absolutely certain that each archesporial cell gives rise to several megaspore mother cells, as well as to trabeculæ and tapetum. In the microsporangium, too, the trabeculæ alone outnumber the archesporial cells (*cf. figs. 31, 46*); and their extreme irregularity and frequent branching and anastomosis make their origin each from a single cell exceedingly improbable.

[*To be concluded.*]



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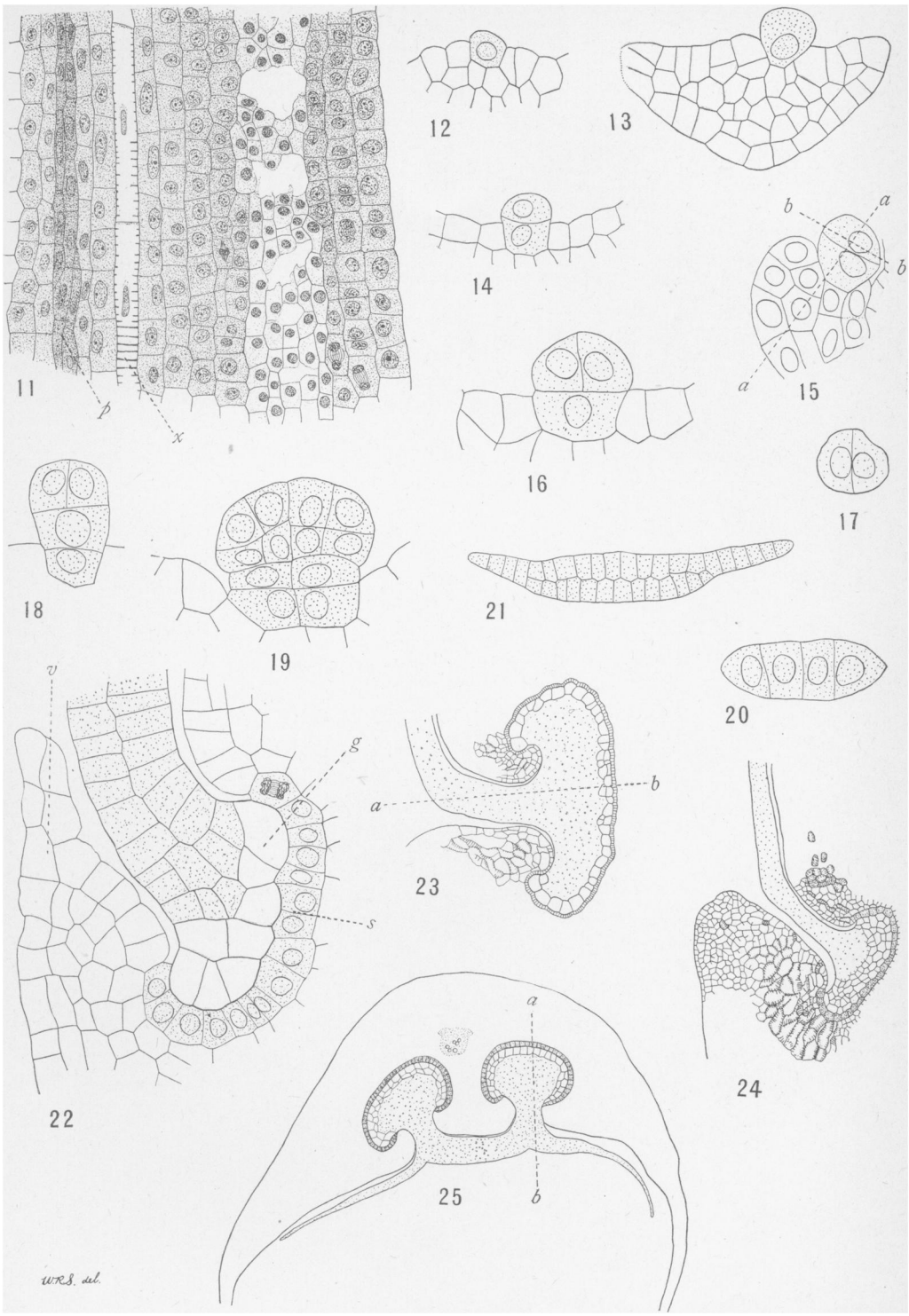

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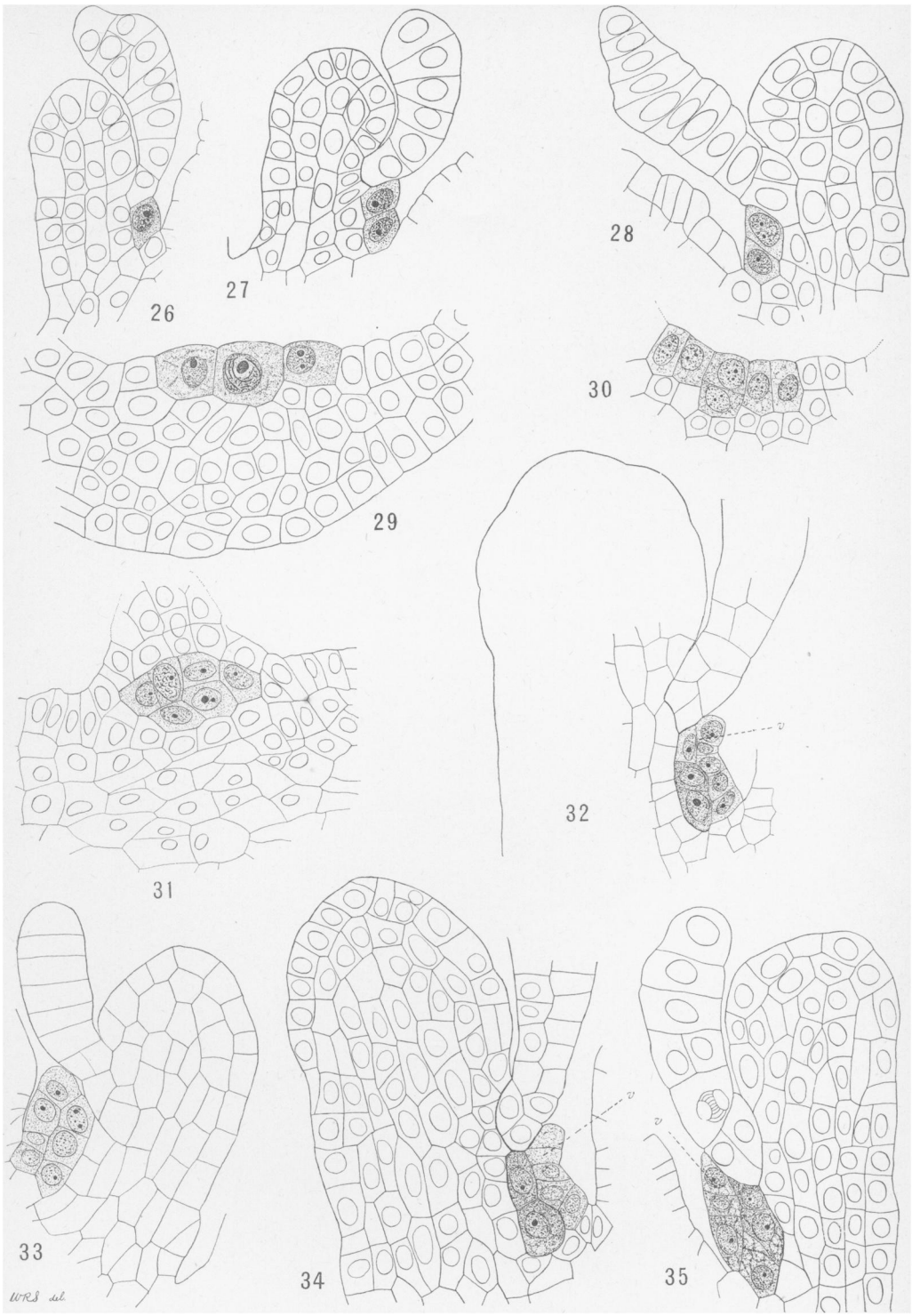

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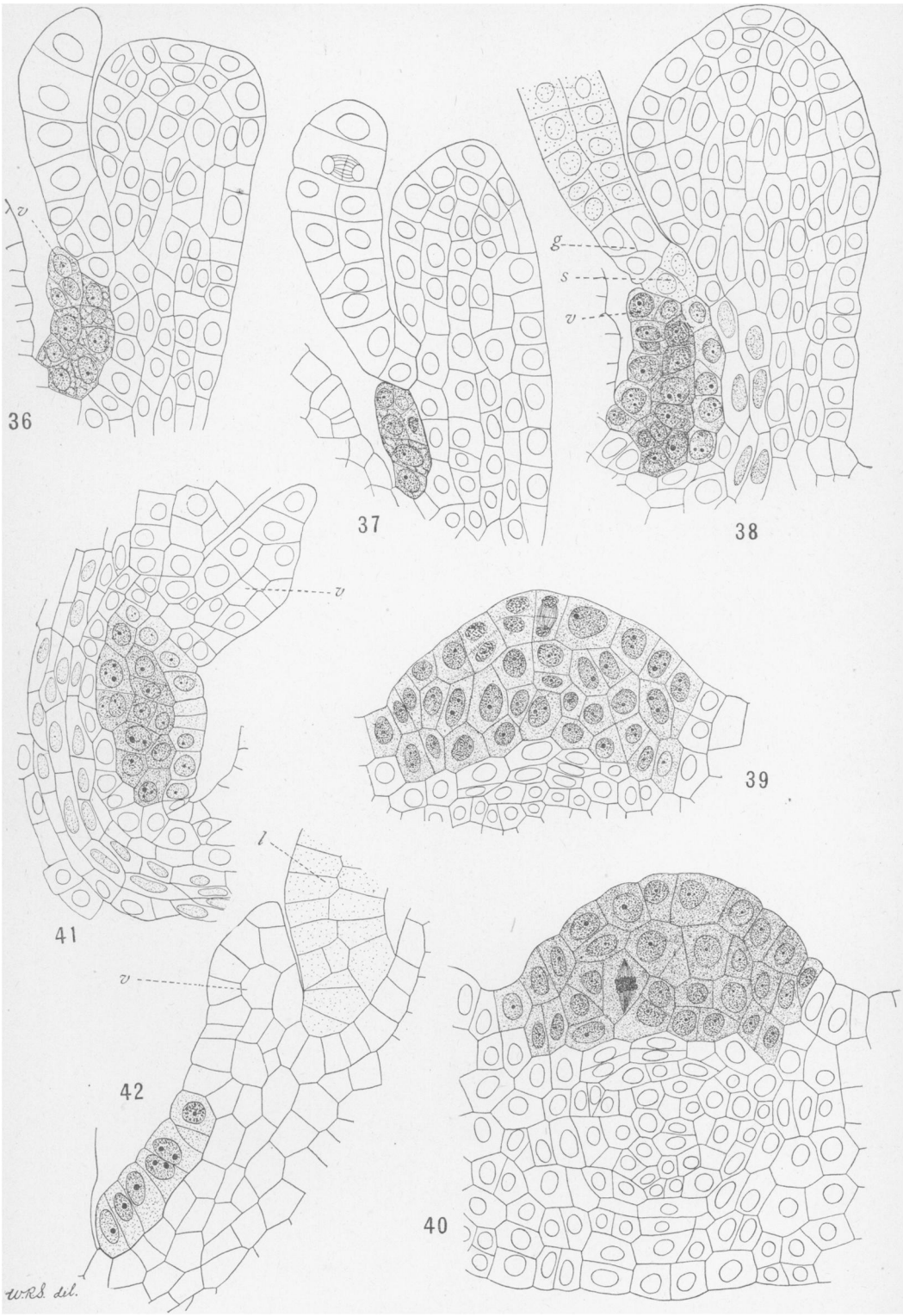

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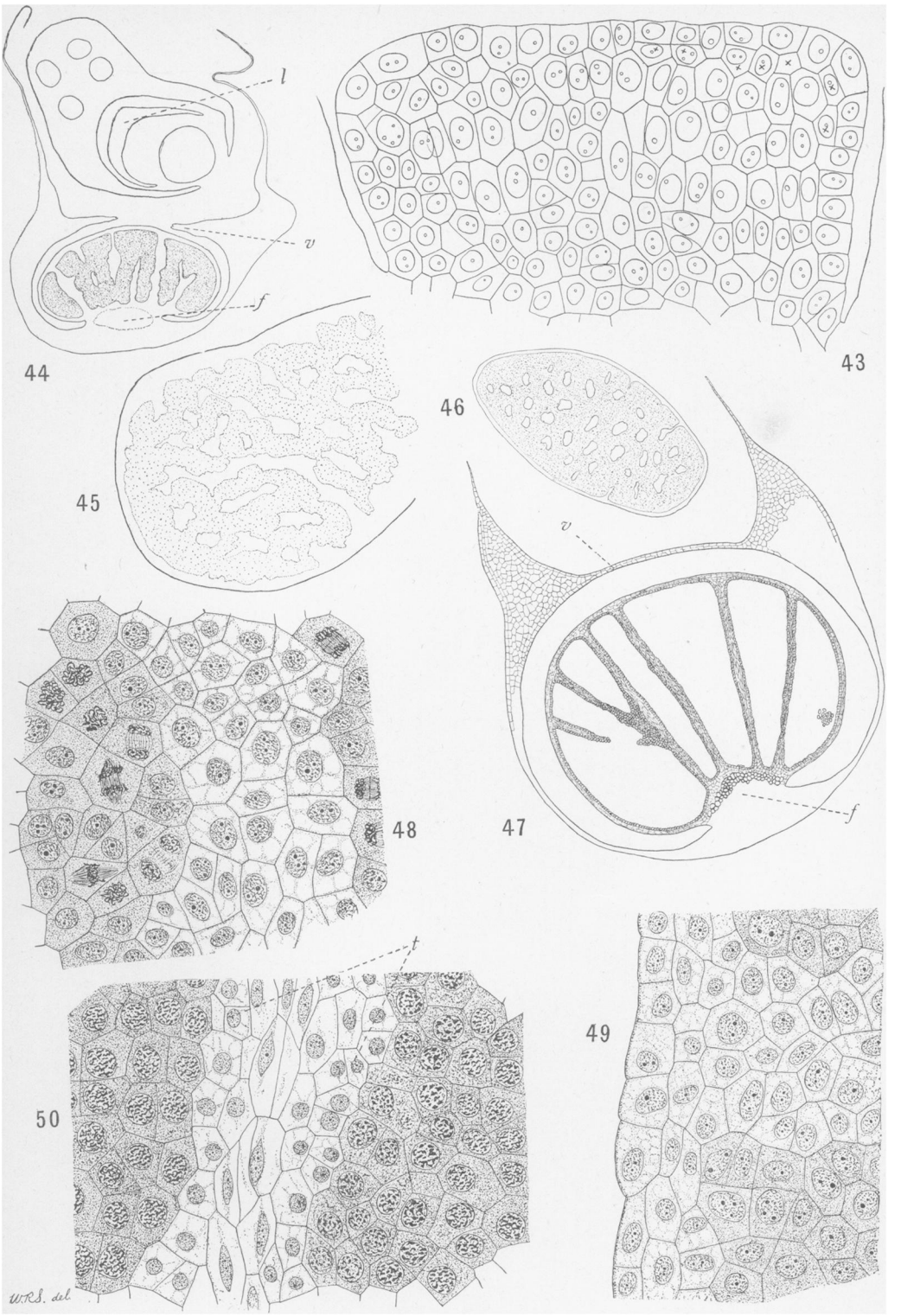

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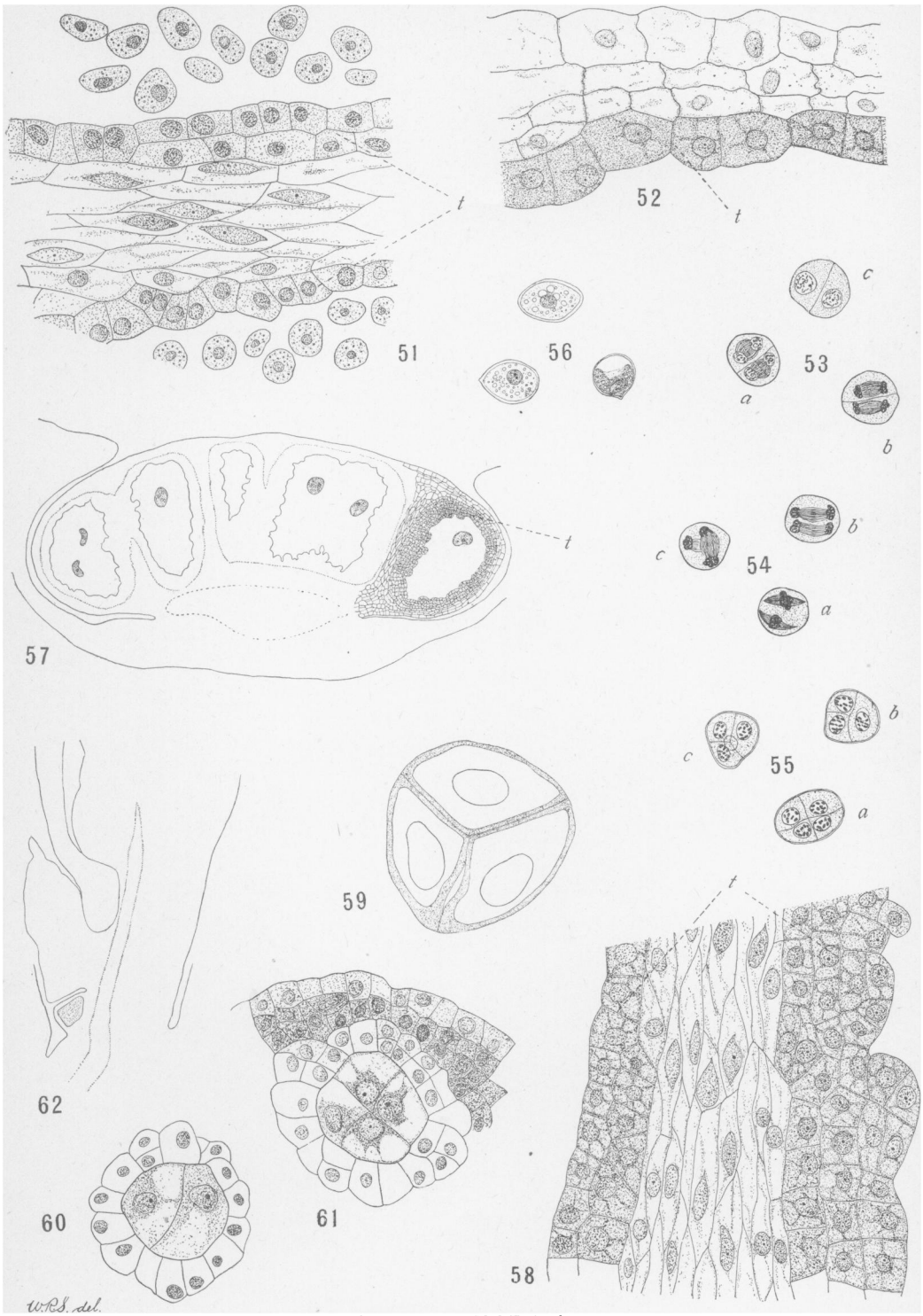

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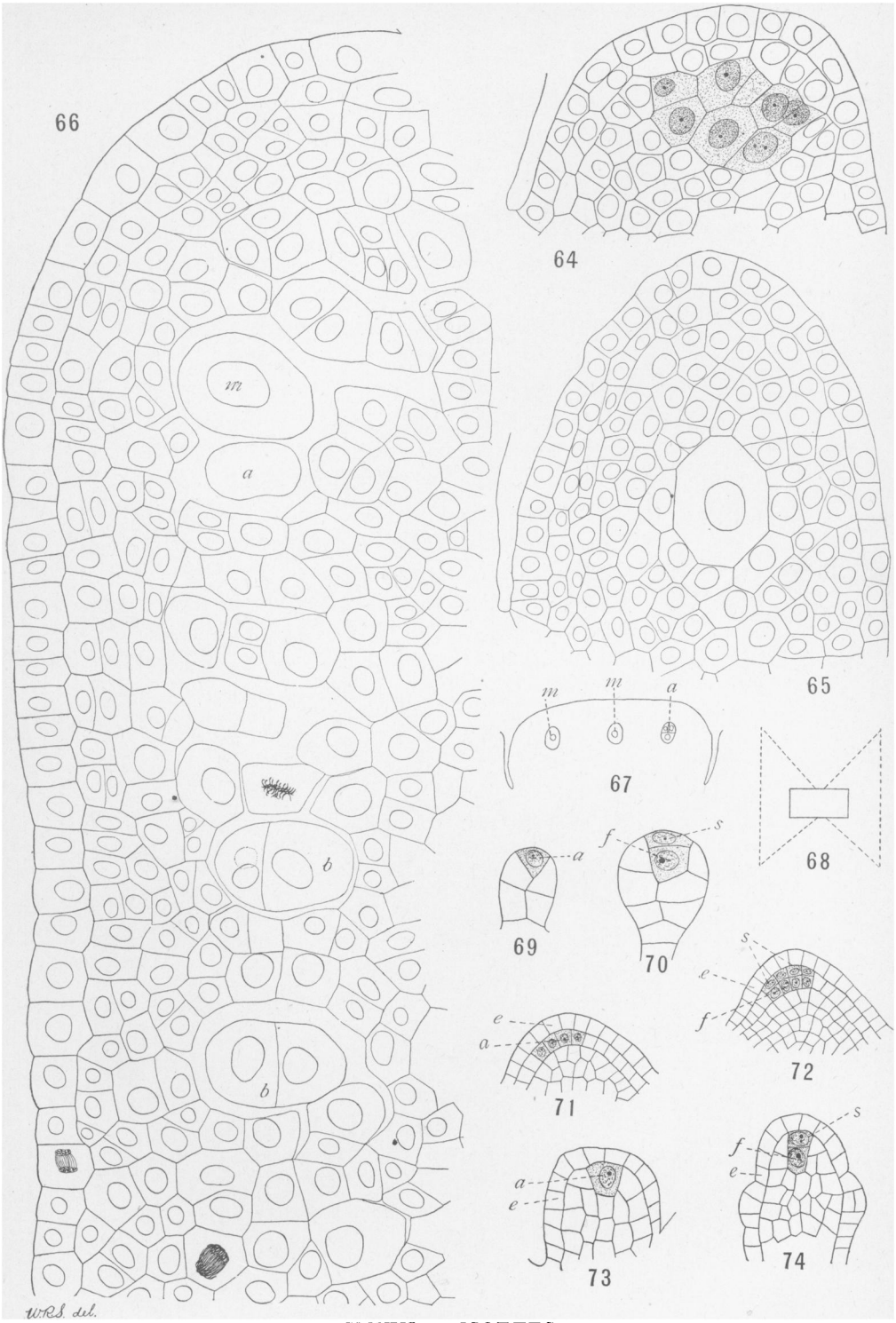

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